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(54) Title: FORMULATION TO ENHANCE BIOAVAILABILITY OF BIOACTIVE MATRIALS AND PREPARATION METHOD THEREOF

(57) Abstract: The present invention relates to compositions and formulations to enhance bioavailability of bioactive materials and preparation method thereof. More particularly, the present invention relates to a composition comprising at least one monoglyceride, at least one emulsifier, organic solvents and aqueous solution and a liquid and powder formulation prepared by adding bioactive material with a low bioavailabiliy to enhance bioavailability of bioactive materials and to acquire high encapsulation efficiency of the bioactive material and high storage stability for a long period of time and preparation method thereof.

FORMULATION TO ENHANCE BIOAVAILABILITY OF BIOACTIVE MATERIALS AND PREPARATION METHOD THEREOF

TECHNICAL FIELD

The present invention relates to a composition and a formulation to enhance bioavailability of bioactive materials and preparation method thereof. More particularly, the present invention relates to a composition comprising at least one monoglyceride, at least one emulsifier, organic solvents and aqueous solution and a liquid or powder formulation prepared by adding bioactive material with a low bioavailability to enhance bioavailability of bioactive materials and to acquire high encapsulation efficiency of the bioactive material and high storage stability for a long period of time and preparation method thereof.

BACKGROUND OF THE INVENTION

Solubilization of materials can be applied to a variety of fields. In chemical processing where their catalysts, reactants, intermediates, etc. are water-insoluble materials, the solubilization of these materials will affect the yield and the direction of a given reaction. Numerous Pharmaceutical compounds with good physiological activities are known to be insoluble in water and thus they have relatively low bioavailabilities. Because the

solubilization of these Pharmaceutical compounds can simplify administration routes and increase pharmaceutical effects, the solubilization technology is essential in commercializing water-insoluble pharmaceuticals, and therefore extensive worldwide studies have been focused on developing the solubilization technology. Cyclosporin and paclitaxel (Taxol®), for instance, are good Examples of water-insoluble Pharmaceutical compounds that cannot be administered alone due to their low solubilities. The solubilizing technology, therefore, has been developed simultaneously with the development of these Pharmaceutical compounds themselves. Cyclosporin and paclitaxel are commercially available as pre-concentrates of Cremophor emulsion. These formulations can spontaneously form microemulsion upon dispersion in water (U.S. Pat. No. 5,438,072).

In general, solubilizing formulations include emulsion or liposome, which uses lipid as a medium, and a polymeric nanoparticle or polymeric micelle, which uses a polymer as a medium (Langer, R. Nature, 392, 5-10, 1998). Of these, the formulations using lipid as a medium are relatively advantageous in that their raw materials are biocompatible and thus they can be widely applied to medical fields including drug delivery systems. In particular, the emulsions are heterogeneous mixture of oil and water by the use of emulsifiers. The oil-in-water type emulsions, composed of oil components dispersed in water, are widely used in solubilizing water-insoluble pharmaceutical compounds. Liposome formulations consist of spherical lipid vesicles with lipid bilayers and water-insoluble Pharmaceutical compounds are enclosed within the lipid bilayer.

U.S. Pat. No. 5,531,925 discloses Cubosome®, another type of formulation using lipid as a solubilization medium, which was first developed by Swedish scientists in early 1990s. Cubosome® is prepared by dispersing the hydrated lipid cubic phase in water. The interior of Cubosome® comprises cubic phase wherein lipid and water components constitute continuous but separate three-dimensional channels, and there exists an interface between lipid headgroup and water. Therefore, Cubosome® could be advantageous over the conventional emulsion type or liposome type formulations, which only allow solubilization of hydrophobic and hydrophilic Pharmaceutical compounds, respectively, in that they can solubilize amphiphilic Pharmaceutical compounds as well as hydrophobic and hydrophilic Pharmaceutical compounds.

Cubosome® can be formed by first forming a very viscous liquid cubic phase by adding water and an emulsifier to monoglyceride, and then by dispersing the mixture in water. Cubosome® has average particle size of as large as several micrometers in diameter with the aid of emulsifiers. Since it is preferential to have submicron-sized particles for the solubilization of Pharmaceutical compounds, it is also possible to obtain submicron-sized particles by applying mechanical forces such as microfluidization.

Preparing submicron-sized Cubosome® particles by means of a mechanical force, however, may result in physicochemical instability of the enclosed materials or the constituting ingredients of the formulations due to high energy and high temperature accompanying the mechanical process. The formulation process may also incur hydrolysis and oxidization of

constituting ingredients because the enclosed materials or the constituting ingredients of the formulations may be vigorously mixed with air during the microfluidization process. Moreover, the dispersed Cubosome® prepared by the microfluidization process may experience the instability of the dispersion system after a prolonged storage and subsequently result in phase separation due to aggregation of particles. Although Cubosome® type formulations have advantageous properties as described above over the conventional type of formulations, they also have disadvantages that they cannot encapsulate the thermally labile pharmaceutical compounds. Also, the physical and chemical stability of the formulation need to be improved greatly to provide a successful drug delivery system.

Peptide and proteins are physiologically active compounds and can be used as therapeutics with specific functions when compared to the molecules with smaller molecular weights.

Proteins, however, cannot be orally administered since they are digested into amino acids by proteases inside the gastro-intestinal tract and lose the physiologically activity. The clearance rate of pharmaceutical proteins is also high in the blood stream when injected, causing great difficulties in administering the protein drugs.

Insulin is a drug that must be administered to the insulin dependent diabetes mellitus (IDDM) patients. Currently, insulin is administered mainly to the patients by subcutaneous injection. Successful oral insulin formulation will bring a revolutionary advancement in the treatment of IDDM. To this end, many pharmaceutical companies and research groups have endeavored to

make an oral insulin formulation with high bioavailability. Due to the limitations in the absorption of insulin in the gastrointestinal tract, however, it is only the beginning stage of the development. Therapeutic peptides including insulin degrades fast by the protease in the intestine resulting in low absorption rate and physiological activity.

To overcome these problems, a variety of peptide delivery systems have been developed. For instance, insulin was encapsulated inside liposomes or polymeric microspheres or administered together with protease inhibitors to prohibit the degradation of insulin. The oral bioavailability could not be enhanced much, however, since the carriers such as liposomes have a relatively low encapsulation efficiency, and the insulin can also degrade during the encapsulation process.

Also, other proteins beside insulin in the intestine cannot be digested causing adverse side effects if the protease inhibitors are administered simultaneously with insulin.

To solve the above-mentioned problems, a variety of drug delivery systems are being developed. Cyclosporin is the only peptide drug that can be administered orally. Cyclosporin is commercially available as pre-concentrates of Cremophor emulsion which can spontaneously form microemulsion upon dispersion in water (U.S. Pat. No. 5,438,072). There are no other oral peptide or protein formulations in the market, however, excepting the oral cyclosporin formulation. The main difficulty arises from the fact that peptide or protein is hard to be encapsulated inside a carrier, can be easily denatured and loses the pharmaceutical activity upon the storage

even if it can be encapsulated in the carrier.

The present inventors have developed a solubilizing composition for insoluble drugs comprising a monoglyceride, an emulsifier and an organic solvent (Korea patent application 2000-12465) to overcome the problems of poor oral bioavailability.

The above solubilizing composition solubilizes various compounds, especially insoluble and amphiphilic substances effectively. This liquid formulation can be easily dispersed in water, by shaking or vortexing, to form a dispersion of particles of less than 500 nm. The above solubilizing composition can encapsulate most of the drugs efficiently even though the encapsulating efficiency depends on many factors including the molecular weight or hydrophobicity of the drugs. Peptides or proteins, however, cannot be solubilized in the above solubilizing composition since they do not mix with any of the components and forms precipitations.

There is a great need for a successful oral peptide formulation that can encapsulate peptides, proteins, water-soluble polymers effectively since the conventional Cubosome® has a stability problem, and the solubilizing composition of Korea patent application 2000-12465 cannot solubilize peptides or proteins.

SUMMARY OF THE INVENTION

After intensive studies to solve the above problems, the inventors of

the present invention finally succeeded in preparing a homogeneous liquid composition comprising monoglycerides, emulsifiers, organic solvents, and water for enhanced bioavailability of bioactive compounds. The present inventors also prepared a composition for enhanced bioavailability of bioactive compounds by adding and aqueous solution of peptide, protein or water-soluble polymers in the mixture of monoglycerides, emulsifier and organic solvent.

Therefore, it is the object of the present invention to provide a formulation for enhanced bioavailability of bioactive compounds including peptides, proteins and drugs for injection or external application by solubilizing and delivering the bioactive compounds efficiently inside the body and the preparation method thereof.

Also, another object of the present invention is to provide a powder composition for enhanced bioavailability of bioactive compounds prepared from the above liquid composition for enhanced bioavailability of bioactive compounds.

Yet another object of the present invention is to provide a powder formulation for enhanced bioavailability of bioactive compounds prepared by adding at least one bioactive compound with a low bioavailability into the above composition for enhanced bioavailability of bioactive compounds.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a liquid composition for enhanced bioavailability of bioactive compounds comprising 9~90% by weight of at least one monoglyceride to increase bioavailability, 0.01~80 % by weight of at least one emulsifier, 0.01~ 10 % by weight of aqueous solution to solubilize the bioactive compounds with a low bioavailability and 0.001~90.9 % by weight of at least one organic solvent.

The above monoglycerides are preferably selected from the group consisting of one or more saturated or an unsaturated monoglycerides having 10 ~ 22 carbon atoms in the hydrocarbon chain. The content of the monoglycerides in the total amount of the liquid formulation is preferably 9 ~ 90 % by weight. Monoglycerides is selected preferably from a group of consisting of monoolein, monopalmitolein, monomyristolein, monoelaidin, and monoerucin, and more preferably monoolein.

The emulsifier is preferably selected from the group consisting of a phospholipid, a sphingolipid, a non-ionic surfactant, an anionic surfactant, a cationic surfactant and bile acid. The content of the emulsifier in the total amount of the liquid formulation is preferably 0.01 ~ 80 % by weight.

The phospholipid used as the above emulsifier is preferably selected from the group consisting of a phosphatidylcholine (PC) and its derivative, a phosphatidylethanolamine (PE) and its derivative, a phosphatidylserine (PS) and its derivative and a polymeric lipid wherein a hydrophilic polymer is conjugated to the lipid headgroup.

The sphingolipid used as the above emulsifier is preferably selected from the group consisting of a ceramide, a cerebroside and a sphingomyelin.

The non-ionic surfactant used as the above emulsifier is preferably selected from the group consisting of a poloxamer (Pluronic: polyoxyethylene-polyoxypropylene copolymer), a sorbitan ester (Span), a polyoxyethylene sorbitan (Tween) and a polyoxyethylene ether (Brij).

The anionic surfactant used as the above emulsifier is preferably selected from the group consisting of a phosphatidylserine (PS) and its derivative, a phosphatidic acid (PA) and its derivative and sodium dodecyl sulfate (SDS).

The cationic surfactant used as the above emulsifier is preferably selected from the group consisting of 1,2- dioleyl-3-trimethylammonium propane (DOTAP), dimethyldioctadecylammonium bromide (DDAB), N-[1-(1,2-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), 1,2-dioleyl-3-ethylphosphocholine (DOEPC) and 3β-[N-[(N',N'-dimethylamino)ethan]carbamoyl]cholesterol (DC-Chol).

The bile acid used as the above emulsifier is preferably selected from the group consisting of cholic acid, its salt and derivatives; deoxycholic acid, its salt and derivatives; ursodeoxycholic acid, its salt and derivatives; chenocholic acid, its salt and derivatives; and lithocholic acid, its salt and derivatives.

The aqueous solution to solubilize the bioactive compounds with a low bioavailability is preferably selected from the group consisting of water, an acidic solution including hydrochloric acid solution, phosphoric acid and acetic acid solution, a basic solution including sodium bicarbonate solution, and a biochemical buffer solution including phosphate buffered saline (PBS). The

content of the aqueous solution in the total amount of the liquid formulation is preferably 0.01 ~ 10 % by weight.

It is preferable that the organic solvent is selected from the group consisting of alcohol, ethyleneglycol, propylene glycol, polyethyleneglycol, dimethylsulfoxide, and the mixture of these solvents. Also, the organic solvent can be preferably selected from the group consisting of acetone, chloroform, benzene, toluene, acetonitrile, alcohols including octanol and the mixture of these solvents, when the said liquid composition is not used for *in vivo* drug delivery systems. The content of the organic solvent in the total amount of the liquid formulation is preferably 1 ~ 90 % by weight.

Also, it is possible to include 0 ~ 5 % by weight of other additives such as fatty acids, fatty acid esters and fatty acid alcohols in the above liquid composition.

The method of preparing the composition according to the present invention for enhanced bioavailability of bioactive compounds comprises the steps of:

1) preparing a viscous liquid by dissolving at least one monoglyceride and at least one emulsifier in at least one organic solvent containing an aqueous solution capable of solubilizing the bioactive compound; and
2) removing the volatile organic solvent from said viscous liquid in step (1).

It is preferable that the organic solvent in the first step of the above

preparation method is selected from the group consisting of alcohol, ethyleneglycol, propylene glycol, polyethyleneglycol, dimethylsulfoxide, [or] and the mixture of these solvents.

It is preferable that the organic solvent in the second step of the above preparation method is selected from the group consisting of alcohol, ethyleneglycol, propylene glycol, polyethyleneglycol, dimethylsulfoxide, and the mixture of these solvents. Also, the organic solvent can be preferably selected from the group consisting of acetone, chloroform, benzene, toluene, acetonitrule, alcohols including octanol and the mixture of these solvents, when the said liquid composition is not used for *in vivo* drug delivery systems. The content of the organic solvent in the total amount of the liquid formulation is preferably 0.001 ~ 90 % by weight.

After the evaporation, a trace of more than 0.001 % of volatile organic solvent of the total composition is still present in the liquid composition since the evaporation process does not require high vacuum. When a solvent mixture is used instead of a single solvent, only the volatile solvent is evaporated and the involatile solvent still leaves in the final composition.

Also, the present invention relates to a powder composition for enhanced bioavailability of bioactive compounds manufactured by lyophilization of the dispersion of the above liquid formulation for enhanced bioavailability of bioactive compounds by adding 0~30% (w/v) of a cryoprotectant.

The method of preparing the powder composition according to the present invention for enhanced bioavailability of bioactive compounds

comprises the steps of:

1) dispersing the above liquid composition in water to prepare the dispersion and

2) lyophilizing the dispersion in step (1) in the presence of cryoprotectant to prepare the powder formulation.

A cryoprotectant may be used to prevent the morphological changes of the particles in the dispersion of the above formulation during lyophilization, and it is preferable to add it less than 30% (w/v) to the liquid formulation. Preferred examples of a cryoprotectant include sugars such as mannitol or trehalose, amino acids such as arginine, and proteins such as albumin.

The liquid and powder composition of the present invention can be easily dispersed in water mediated by such a minimal mechanical aid as a simply shaking with hands. The size of particles is approximately 200 nm in general and can become as large as 500 nm depending on the property of a given emulsifier.

The present invention also provides formulations for enhanced bioavailability of bioactive materials as a drug delivery system.

More particularly, the present invention provides a liquid and a powder formulation for enhanced bioavailability of bioactive materials and the preparation method thereof.

A liquid formulation for enhanced bioavailability of bioactive compounds according to the present invention comprises 0.001 ~ 50 % by weight of at least one bioactive compound with a low bioavailability as an active ingredient, 9 ~ 90 % by weight of at least one monoglyceride

compound, 0.01~80 % by weight of at least one emulsifier, 0.01 ~ 10 % by weight of aqueous solution to solubilize the bioactive compound with a low bioavailability and 0.001 ~ 90 % by weight of organic solvent.

The kinds of monoglyceride compound, emulsifier, aqueous solution and organic solvent are identical as mentioned above.

In the dispersion prepared from the above formulation for enhanced bioavailability of bioactive compounds can encapsulate water-soluble compounds, especially peptides and proteins. The peptides that can be used in the present invention include adrenocorticotropic hormone (ACTH) and its fragments, angiotensin and its related peptides, antibodies and their fragments, antigens and their fragments, atrial natriuretic peptides, bioadhesive peptides, Bradykinins and their related peptides, calcitonins and their related peptides, cell surface receptor protein fragments, chemotactic peptides, cyclosporins, cytokines, Dynorphins and their related peptides, endorphins and β-lidotropin fragments, enkephalin and their related proteins, enzyme inhibitors, fibronectin fragments and their related peptides, gastrointestinal peptides, growth hormone releasing peptides, immunostimulating peptides, insulins and insulin-like growth factors, interleukins, luthenizing hormone releasing hormones (LHRH) and their related peptides, melanocyte stimulating hormones and their related peptides, nuclear localization signal related peptides, neurotensins and their related peptides, neurotransmitter peptides, opioid peptides, oxytocins, vasopressins and their related peptides, parathyroid hormone and its fragments, protein kinases and their related peptides, somatostatins and their related peptides,

substance P and its related peptides, transforming growth factors (TGF) and their related peptides, tumor necrosis factor fragments, toxins and toxoids.

Insulin as a peptide is preferably selected from a group consisting of a bovine pancreatic insulin, a human insulin, a human recombinant insulin, a porcine pancreatic insulin, an arg-insulin, an insulin-biotin, an insulin-FITC, LysPro (Eli Lilly) and a polyethylene glycol-insulin, (PEG-insulin). The content of insulin in the total amount of the liquid formulation is preferably 0.01 ~ 20 % by weight.

Also, the formulation of the present invention can comprise functional peptides such as anticancer peptides including angiostatins, antihypertension peptides, anti-blood clotting peptides, and antimicrobial peptides.

The formulation of the present invention can comprise proteins such as immunoglobulins, angiogenins, bone morphogenic proteins, chemokines, colony stimulating factors (CSF) and their related proteins, cytokines, growth factors, interferons, interleukins, leptins, leukemia inhibitory factors, stem cell factors, transforming growth factors and tumor necrosis factors.

The above formulations can enclose a hydrophilic, a hydrophobic and an amphiphilic compound, especially a compound with a high molecular weight as a Pharmaceutical compound. Examples of these Pharmaceutical compounds that can be used in the present invention are antivirals, steroidal anti-inflammatory drugs (SAID), non-steroidal anti-inflammatory drugs (NSAID), antibiotics, antifungals, vitamins, hormones, retinoic acid, prostaglandins, prostacyclins, anticancer drugs, antimetabolitic drugs, miotics, cholinergics, adrenergic antagonists, anticonvulsants, antianxiety agents,

major tranquilizers, antidepressants, anesthetics, analgesics, anabolic steroids, estrogens, progesterones, glycosaminoglycans, polynucleotides, immunosuppressants and immunostimulants.

The above formulations can solubilize a hydrophilic and an amphiphilic polymer. Examples of these polymers that can be used in the present invention are chitosan, alginic acid, alginate, hyaluronic acid, hyaluronate, polyethylene glycol and other functional polymers.

Also it is possible to include 0 ~ 5 % by weight of other additives such as fatty acids, fatty acid esters and fatty acid alcohols in the above liquid formulation.

The preparation method of the above liquid formulation for enhanced bioavailability of bioactive compounds comprises the steps of:

1) preparing a viscous liquid by dissolving at least one bioactive compound as an active ingredient, at least one monoglyceride and at least one emulsifier in at least one organic solvent containing an aqueous solution for solubilizing the bioactive compound; and
2) removing the volatile organic solvent from said viscous liquid in step (1).

After the evaporation, a trace of more than 0.001 % of volatile organic solvent of the total formulation is still present in the liquid formulation since the evaporation process does not require high vacuum. When a solvent mixture is used instead of a single solvent, only the volatile solvent is evaporated leaving involatile solvent in the final formulation.

It is preferable that the organic solvent in the first step of the above preparation method is selected from the group consisting of alcohol, ethyleneglycol, propylene glycol, polyethyleneglycol, dimethylsulfoxide, and the mixture of these solvents.

It is preferable that the organic solvent in the second step of the above preparation method is selected from the group consisting of alcohol, ethyleneglycol, propylene glycol, polyethyleneglycol, dimethylsulfoxide, and the mixture of these solvents. The organic solvent can also be selected from the group consisting of acetone, chloroform, benzene, toluene, acetonitrule, alcohols including octanol when the said liquid composition is not used for *in vivo* drug delivery systems. The content of the organic solvent in the total amount of the liquid formulation is preferably 0.001 ~ 90 % by weight.

Also, the present invention provides a powder formulation for enhanced bioavailability of bioactive compounds manufactured by lyophilization of the dispersion of the above liquid formulation by adding 0~30% (w/v) of a cryoprotectant.

The preparation method of the above powder formulation comprises the steps of:

1) dispersing the above liquid formulation in water to prepare the dispersion; and
2) lyophilizing said dispersion in step (1) in the presence of cryoprotectant.

A cryoprotectant may be used to prevent the morphological changes in the dispersion of the above formulation during lyophilization, and it is

preferred to add it less than 30% (w/v) to the liquid formulation. Preferred examples of a cryoprotectant include sugars such as mannitol or trehalose, amino acids such as arginine, and proteins such as albumin.

The liquid and powder formulation of the present invention can be easily dispersed in water mediated by such a minimal mechanical aid as a simply shaking with hands. The size of particles is approximately 200 nm in general and can become as large as 500 nm depending on the property of a given pharmaceutical compounds or an emulsifier. Moreover, the constituting ingredients of and pharmaceutical compound in the particles are not degraded since a strong mechanical force is not required in generating the particles.

In case the formulation according to the present invention is administered orally, the above powder formulation dispersed easily in water before the intake. Also, the powder formulation can be dispersed in water for injection.

The formulations according to the present invention can be stored at room temperature in a sealed state for a long period of time without undergoing phase separation or the change in properties of the formulations. When a long-term storage is required, the powder formulation is desirable because it does not contact with an organic solvent or moisture.

Moreover, the formulations of the present invention can be used as an drug delivery system since the loading efficiency is as high as 50 ~ 100 % with the simple preparation protocol. When applying these formulations in drug delivery system, it is preferred to use various administration routes

including oral administration, buccal administration, mucosal administration, nasal administration, intra peritoneal administration, subcutaneous injection, intra muscular injection, transdermal administration and intravenous injection, and more preferably an oral administration.

This invention is explained in more detail based on the following Examples but they should not be construed as limiting the scope of this invention.

Example 1. Preparation of Liquid Formulation Containing Bovine Serum Albumin

1-1. Preparation of the Liquid Formulation

In 400 μl of methanol, 200 mg of monoolein and 40 mg of Pluronic F-68 were dissolved. The mixture was heated up to 50 °C to accelerate the dissolution when necessary. After mixing 4 μl of 200 mg/ml bovine serum albumin (BSA) aqueous solution and 960 mg of propylene glycol, the mixture was added to the above monoolein solution and stirred to prepare a homogeneous liquid solution. Methanol in the formulation was evaporated completely by purging with oxygen-free nitrogen gas to prepare the viscous liquid formulation.

1-2. Property Analysis of thus prepared Liquid Formulation

The size and the polydispersity of the emulsion particles were measured after diluting 10 μl of thus obtained liquid formulation by adding 3

ml of phosphate buffered saline (PBS) at pH 7.4. The size the polydispersity of the emulsion particles were measured by Photon Correlation spectroscopy (QELS method) using Malvern Zetasizer (Malvern Instruments Limited, England), and the polydispersity is a variance of the particle size in a logarithmic scale of a log-normal distribution function of the particle size. The average particle size and polydispersity were obtained by measuring a given formulation three times (Orr, Encyclopedia of emulsion technology, 1, 369-404, 1985). This method was used in measuring the particle size and the polydispersity throughout the following Examples.

The above liquid formulations were dispersed well in water, and the average particle size and polydispersity were about 296.2 nm and 0.164, respectively.

Two hundred microliters of the liquid formulation was dispersed in 1.8 ml of water. The dispersion was transferred into the retentate vial of Centricon® (MWCO 300,000, Millipore Corporation, Bedford, MA, USA) and centrifuged at 1000 x g for 30 min. The concentrations of total and unencapsulated protein were calculated by analyzing the protein concentrations in the dispersion and in the filtrate, respectively by the BCA method. Encapsulation efficiency of the protein was also calculated by the ratio between the concentrations of the encapsulated protein and the total protein. The encapsulation efficiency was *ca.* 80 %.

Example 2. Preparation of Powder Formulation Containing Bovine Serum Albumin

In 0.1 ml of the liquid formulation Containing Bovine Serum Albumin obtained in Example 1, 1 ml of 5 % trehalose aqueous solution was added and solubilized completely by shaking. The mixture was lyophilized to prepare a powder formulation. The average particle size and polydispersity of the dispersion of the powder formulation were determined after dispersing approximately 3 mg of the above powder formulation in 3 ml of water or 0.01 M sodium deoxycholate as described in Example 1-2.

The above powder formulation was dispersed well in water, and, as indicated in the following table 1, the average particle size and polydispersity of the dispersion of the powder formulation were 483.6 nm and 0.461, respectively, in water and 170.0 nm and 0.276, respectively, in 0.01 M sodium deoxycholate.

Table 1

Redispersed solution	size (nm)	Polydispersity
Water	483.6	0.461
0.01 M sodium deoxycholate	170.0	0.276

Example 3. Preparation of Liquid Formulation Containing Tetanus Toxoid

1-1. Preparation of the Liquid Formulation

In 120 μl of ethanol, 20 mg Pluronic F-68 was dissolved. The mixture

was heated up to 50 °C to accelerate the dissolution when necessary. After mixing 40 μl of the 5.376 mg/ml tetanus toxoid aqueous solution and 280 mg of propylene glycol, 100 mg of monoolein and the above Pluronic F-68/ethanol solution was added to the above mixture of tetanus toxoid and propylene glycol and stirred to prepare a homogeneous liquid solution. Ethanol in the formulation was evaporated completely by purging with oxygen-free nitrogen gas to prepare the viscous liquid formulation.

3-2. Property Analysis of thus prepared Liquid Formulation

The average particle size and polydispersity of the dispersion of the liquid formulation were determined after dispersing approximately 10 μl or 75 μl of the above liquid formulation in 3 ml of water or 2.5 ml of 0.01 M sodium deoxycholate, respectively, as described in Example 1-2.

The above formulation was dispersed well in water, and the average particle size and polydispersity of the dispersion of the liquid formulation were 303.9 nm and 0.185, respectively, in water and 175.2 nm and 0.377, respectively, in 0.01 M sodium deoxycholate. The encapsulation efficiency of tetanus toxoid was determined as described in Example 1-2 except that the molecular weight cut off of the Centricon® was 1,000,000, and was 80 ~ 85%.

Table 2

Dispersing solution	size (nm)	Polydispersity
water	303.9	0.185
0.01 M sodium deoxycholate	175.2	0.377

Example 4. Preparation of Liquid Formulation Containing Pneumococcal surface adhesin A (PsaA)

4-1. Preparation of the Liquid Formulation

The pneumococcal surface adhesin A (hereafter referred to "PsaA") dissolved in water was concentrated by using Sep-Pak Cartridges (Waters) to 0.5 mg/ml in methanol. In 1 ml of methanol, 10 mg of monoolein and 2 mg Pluronic F-68 or Pluronic F-127 were dissolved. The mixture was heated to 50 °C to accelerate the dissolution when necessary. After mixing 0 μl, 50 μl, or 500 μl of 0.5 mg/ml PsaA in methanol solution and 48 mg of propylene glycol, the mixture was added to the above monoolein solution and stirred to prepare a homogeneous liquid solution. Methanol in the formulation was evaporated completely by purging with oxygen-free nitrogen gas to prepare the viscous liquid formulation.

4-2. Property Analysis of thus prepared Liquid Formulation

The average particle size and polydispersity of the dispersion of the liquid formulation were determined after dispersing approximately 10 μl of the above liquid formulation in 3 ml of water, respectively, as described in Example 1-2.

The above formulation was dispersed well in water, and the average particle size and polydispersity of the dispersion of the liquid formulation are summarized in Table 3.

The encapsulation efficiency of PsaA was determined as described in Example 1-2 and was *ca.* 50 %.

Example 5. Preparation of Powder Formulation Containing PsaA

In 0.1 ml of the liquid formulation Containing PsaA obtained in Example 1, 1 ml of 5 % trehalose aqueous solution was added and solubilized completely by shaking. The mixture was lyophilized to prepare a powder formulation. The average particle size and polydispersity of the dispersion of the powder formulation were determined after dispersing approximately 3 mg of the above powder formulation in 3 ml of water or 0.01 M sodium deoxycholate as described in Example 1-2.

The above powder formulation was dispersed well in water, and the average particle size and polydispersity of the dispersion of the powder formulation are summarized in Table 3.

The encapsulation efficiency of PsaA was determined as described in Example 1-2 and was *ca.* 100 %.

Table 3

	Formulation	Emulsifier	Amount of PsaA solution (μl)	Size (nm)	Polydispersity
Example 4	Liquid Formulation	Pluronic F68	0	231.6	0.055
			50	228.2	0.175
			500	220.1	0.063
		Pluronic F127	0	233.2	0.201
			50	214.5	0.097
			500	237.8	0.244
Example 5	Powder Formulation	Pluronic F68	0	244.6	0.145
			50	227.0	0.098
			500	217.5	0.123
		Pluronic F127	0	243.0	0.152
			50	228.3	0.092
			500	229.7	0.138

Example 6. Preparation of Liquid Formulation Containing Calcitonin

6-1. Preparation of the Liquid Formulation

In the mixed solvent of 280 mg of anhydrous ethanol and 280 mg of propylene glycol, 20 mg of Pluronic F-127 was solubilized completely. The mixture was heated to 50 °C to accelerate the dissolution when necessary. After adjusting pH of the mixed solvent to 3.0 ~ 4.0 by adding 1 N hydrochloride solution, 8.4 mg of calcitonin (Thyrocalcitonin = calcitonin from bovine thyroid glands, F.W. 3593.0, 0.108 I.U./mg) was added and stirred. When the solution became clear, 100 mg of monoolein was added and stirred

until clear. Ethanol in the formulation was evaporated completely by purging with oxygen-free nitrogen gas to prepare the viscous liquid formulation.

6-2. Property Analysis of thus prepared Liquid Formulation

The average particle size and polydispersity of the dispersion of the liquid formulation were determined after dispersing approximately 10 μl of the above liquid formulation in 3 ml of water, respectively, as described in Example 1-2.

The above formulation was dispersed well in water, and the average particle size and polydispersity of the dispersion of the liquid formulation were 209.2 nm and 0.279, respectively.

The encapsulation efficiency of calcitonin was determined as described in Example 1-2 and was *ca.* 50 %.

Example 7. Preparation of Liquid Formulation Containing Growth Hormone

7-1. Preparation of the Liquid Formulation

A liquid insulin formulation was obtained by using the same procedure as described in Example 6 except that 3.0 mg of a growth hormone (somatotropin, from human pituitaries, 2 I.U./mg, Sigma) was used instead of calcitonin.

7-2. Property Analysis of thus prepared Liquid Formulation

The average particle size and polydispersity of the dispersion of the

liquid formulation were determined after dispersing approximately 10 μl of the above liquid formulation in 3 ml of water, respectively, as described in Example 1-2.

The above formulation was dispersed well in water, and the average particle size and polydispersity of the dispersion of the liquid formulation were 234.9 nm and 0.224, respectively.

The encapsulation efficiency of PsaA was determined as described in Example 1-2 and was *ca.* 70 %.

Example 8. Preparation of Liquid Formulation Containing Blue Dextran

8-1. Preparation of the Liquid Formulation

In 360 μl of ethanol, 60 mg of Pluronic F-68 was dissolved. The mixture was heated to 50 °C to accelerate the dissolution when necessary. After mixing 300 μl of the 40 mg/ml of blue dextran (molecular weight 2,000,000, Sigma) aqueous solution and 840 mg of propylene glycol, 300 mg of monoolein and the above Pluronic F-68/ethanol solution were added to the above mixture and stirred to prepare a homogeneous liquid solution. Ethanol in the formulation was evaporated completely by purging with oxygen-free nitrogen gas to prepare the viscous liquid formulation.

8-2. Property Analysis of thus prepared Liquid Formulation

The average particle size and polydispersity of the dispersion of the liquid formulation were determined after dispersing approximately 10 μl of the

above liquid formulation in 3 ml of water, respectively, as described in Example 1-2.

The above formulation was dispersed well in water, and the average particle size and polydispersity of the dispersion of the liquid formulation were 333.2 nm and 0.316, respectively.

The encapsulation efficiency of blue dextran was determined as described in Example 1-2 and was *ca.* 90 %.

As shown in Examples 1 ~ 8, the dispersion of the liquid and powder formulation according to the present invention has a particle size of less than 500 nm, a high solubilizing power of drugs and a high encapsulation efficiency of drugs.

EFFECT OF THE INVENTION

As described above, the formulations according to the present invention can solubilize and encapsulate bioactive compounds with a low bioavailability such as peptides or proteins stably and also generate homogeneous particles less than 500 nm when dispersed in water. Moreover, the formulations can be easily dispersed in water without any mechanical aid, and problems such as phase separation, hydrolysis and oxidation, during long-term storage, can be prevented thus being suitable for use in drug delivery system.

CLAIMS

1 A liquid composition for enhanced bioavailability of bioactive compounds, comprising 9 ~ 90 % by weight of at least one monoglyceride compound as an uptake enhancer, 0.01~80 % by weight of at least one emulsifier, 0.01 ~ 10 % by weight of aqueous solution to solubilize the bioactive compound and 0.001 ~ 90 % by weight of at least one organic solvent.

2 The liquid composition for enhanced bioavailability of bioactive compounds according to claim 1, wherein said monoglyceride is selected from the group consisting of a saturated or an unsaturated monoglyceride having 10 ~ 22 carbon atoms in the hydrocarbon chain.

3 The liquid composition for enhanced bioavailability of bioactive compounds according to claim 2, wherein said monoglyceride compound is selected from the group consisting of monoolein, monopalmitolein, monomyristolein, monoelaidin, and monoerucin.

4 The liquid composition for enhanced bioavailability of bioactive compounds according to claim 1, wherein said emulsifier is selected from the group consisting of a phospholipid, a sphingolipid, a non-ionic surfactant, an anionic surfactant, a cationic surfactant and bile acid.

5 The liquid composition for enhanced bioavailability of bioactive compounds according to claim 4, wherein said phospholipid is selected from the group consisting of a phosphatidylcholine (PC) and its derivative, a phosphatidylethanolamine (PE) and its derivative, a phosphatidylserine (PS) and its derivative and a polymeric lipid wherein a hydrophilic polymer is conjugated to the lipid headgroup.

6 The liquid composition for enhanced bioavailability of bioactive compounds according to claim 4, wherein said sphingolipid is selected from the group consisting of a ceramide, a cerebroside and a sphingomyelin.

7 The liquid composition for enhanced bioavailability of bioactive compounds according to claim 4, wherein said non-ionic surfactant is selected from the group consisting of a poloxamer (Pluronic: polyoxyethylene-polyoxypropylene copolymer), a sorbitan ester (Span), a polyoxyethylene sorbitan (Tween) and a polyoxyethylene ether (Brij).

8 The liquid composition for enhanced bioavailability of bioactive compounds according to claim 4, wherein said anionic surfactant is selected from the group consisting of a phosphatidylserine (PS) and its derivative, a phosphatidic acid (PA) and its derivative and sodium dodecyl sulfate (SDS).

9 The liquid composition for enhanced bioavailability of bioactive compounds according to claim 4, wherein said cationic surfactant is selected from the group consisting of 1,2- dioleyl-3-trimethylammonium propane (DOTAP), dimethyldioctadecylammonium bromide (DDAB), N-[1-(1,2-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), 1,2-dioleyl-3-ethylphosphocholine (DOEPC) and 3β-[N-[(N',N'-dimethylamino)ethan]carbamoyl]cholesterol (DC-Chol).

10 The liquid composition for enhanced bioavailability of bioactive compounds according to claim 4, wherein said bile acid is selected from the group consisting of cholic acid, its salt and derivatives; deoxycholic acid, its salt and derivatives; ursodeoxycholic acid, its salt and derivatives; chenocholic acid, its salt and derivatives; and lithocholic acid, its salt and derivatives.

11 The liquid composition for enhanced bioavailability of bioactive compounds according to claim 1, wherein said organic solvent is selected from the group consisting of alcohol, ethyleneglycol, propylene glycol, polyethyleneglycol, dimethylsulfoxide, acetone, acetonitrile, chloroform, benzene, toluene and the mixture of these solvents.

12 The liquid composition for enhanced bioavailability of bioactive compounds according to claim 1 additionally comprising an additive selected from the group consisting of fatty acids, fatty acid esters and

fatty acid alcohols.

13 The liquid composition for enhanced bioavailability of bioactive compounds according to claim 1, wherein said aqueous solution is selected from a group consisting of buffer solution, acidic solution and basic solution.

14 A method of preparing the liquid composition for enhanced bioavailability of bioactive compounds, wherein said method comprises the steps of:

1) preparing a viscous liquid by dissolving at least one monoglyceride and at least one emulsifier in at least one organic solvent containing an aqueous solution capable of solubilizing the bioactive compound; and
2) removing the volatile organic solvent from said viscous liquid in step (1).

15 A powder composition for enhanced bioavailability of bioactive compounds manufactured by lyophilization of the dispersion of the composition according to claim 1 by adding 0~30% (w/v) of a cryoprotectant.

16 The powder composition for enhanced bioavailability of bioactive compounds according to claim 15, wherein said cryoprotectant is

selected from the group consisting of a sugar, an amino acid and a protein.

17 The powder composition for enhanced bioavailability of bioactive compounds according to claim 16, wherein said sugar is selected from mannitol or trehalose, said amino acid is arginine, and said protein is albumin.

18 The method of preparing the powder composition for enhanced bioavailability of bioactive compounds, wherein said method comprises the steps of:

1) dispersing the liquid composition according to claim 1 in water to prepare the dispersion; and
2) lyophilizing said dispersion in step (1) in the presence of cryoprotectant to prepare the powder formulation.

19 A liquid formulation for enhanced bioavailability of bioactive compounds comprising, 0.001 ~ 50 % by weight of at least one bioactive compound as an active ingredient, 9 ~ 90 % by weight of at least one monoglyceride compound as an uptake enhancer, 0.01~80 % by weight of at least one emulsifier, 0.01 ~ 10 % by weight of aqueous solution to solubilize the bioactive compound and 0.001 ~ 90 % by weight of at least one organic solvent.

20 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 19, wherein said monoglyceride is selected from a group consisting of a saturated or an unsaturated monoglyceride having 10 ~ 22 carbon atoms in the hydrocarbon chain.

21 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 20, wherein said monoglyceride compound is selected from monoolein, monopalmitolein, monomyristolein, monoelaidin, and monoerucin.

22 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 19, wherein said emulsifier is selected from a phospholipid, a sphingolipid, a non-ionic surfactant, an anionic surfactant, a cationic surfactant and bile acid.

23 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 22, wherein said phospholipid is selected from the group consisting of a phosphatidylcholine (PC) and its derivative, a phosphatidylethanolamine (PE) and its derivative, a phosphatidylserine (PS) and its derivative and a polymeric lipid wherein a hydrophilic polymer is conjugated to the lipid headgroup.

24 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 22, wherein said sphingolipid is selected

from the group consisting of a ceramide, a cerebroside and a sphingomyelin.

25 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 22, wherein said non-ionic surfactant is selected from the group consisting of a poloxamer (Pluronic: polyoxyethylene-polyoxypropylene copolymer), a sorbitan ester (Span), a polyoxyethylene sorbitan (Tween) and a polyoxyethylene ether (Brij).

26 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 22, wherein said anionic surfactant is selected from the group consisting of a phosphatidylserine (PS) and its derivative, a phosphatidic acid (PA) and its derivative and sodium dodecyl sulfate (SDS).

27 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 22, wherein said cationic surfactant is selected from the group consisting of 1,2- dioleyl-3-trimethylammonium propane (DOTAP), dimethyldioctadecylammonium bromide (DDAB), N-[1-(1,2-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), 1,2-dioleyl-3-ethylphosphocholine (DOEPC) and 3β-[N-[(N',N'-dimethylamino)ethan]carbamoyl]cholesterol (DC-Chol).

28 The liquid formulation for enhanced bioavailability of bioactive

compounds according to claim 22, wherein said bile acid is selected from the group consisting of cholic acid, its salt and derivatives; deoxycholic acid, its salt and derivatives; ursodeoxycholic acid, its salt and derivatives; chenocholic acid, its salt and derivatives; and lithocholic acid, its salt and derivatives.

29 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 19, wherein said organic solvent is selected from the group consisting of alcohol, ethyleneglycol, propylene glycol, polyethyleneglycol, dimethylsulfoxide, acetone, acetonitrile, chloroform, benzene, toluene and the mixture of these solvents.

30 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 19, additionally comprising an additive selected from the group consisting of fatty acids, fatty acid esters and fatty acid alcohols.

31 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 19, wherein said aqueous solution is selected from the group consisting of buffer solution, acidic solution and basic solution.

32 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 19, wherein said bioactive compound is

selected from the group consisting of peptides such as adrenocorticotropic hormone (ACTH) and its fragments, angiotensin and its related peptides, antibodies and their fragments, antigens and their fragments, atrial natriuretic peptides, bioadhesive peptides, Bradykinins and their related peptides, calcitonins and their related peptides, cell surface receptor protein fragments, chemotactic peptides, cyclosporins, cytokines, Dynorphins and their related peptides, endorphins and β-lidotropin fragments, enkephalin and their related proteins, enzyme inhibitors, fibronectin fragments and their related peptides, gastrointestinal peptides, growth hormone releasing peptides, immunostimulating peptides, insulins and insulin-like growth factors, interleukins, luthenizing hormone releasing hormones (LHRH) and their related peptides, melanocyte stimulating hormones and their related peptides, nuclear localization signal related peptides, neurotensins and their related peptides, neurotransmitter peptides, opioid peptides, oxytocins, vasopressins and their related peptides, parathyroid hormone and its fragments, protein kinases and their related peptides, somatostatins and their related peptides, substance P and its related peptides, transforming growth factors (TGF) and their related peptides, tumor necrosis factor fragments, toxins and toxoids and functional peptides such as anticancer peptides including angiostatins, antihypertension peptides, anti-blood clotting peptides, and antimicrobial peptides.

33 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 19, wherein said bioactive compound is selected from the group consisting of proteins such as immunoglobulins, angiogenins, bone morphogenic proteins, chemokines, colony stimulating factors (CSF), cytokines, growth factors, interferons, interleukins, leptins, leukemia inhibitory factors, stem cell factors, transforming growth factors and tumor necrosis factors.

34 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 19, wherein said bioactive compound is selected from the group consisting of antivirals, steroidal anti-inflammatory drugs (SAID), non-steroidal anti-inflammatory drugs (NSAID), antibiotics, antifungals, vitamins, hormones, retinoic acid, prostaglandins, prostacyclins, anticancer drugs, antimetabolitic drugs, miotics, cholinergics, adrenergic antagonists, anticonvulsants, antianxiety agents, major tranquilizers, antidepressants, anesthetics, analgesics, anabolic steroids, estrogens, progesterones, glycosaminoglycans, polynucleotides, immunosuppressants and immunostimulants.

35 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 19, wherein said bioactive compound is selected from the group consisting of cationic, neutral and anionic polymers.

36 The liquid formulation for enhanced bioavailability of bioactive compounds according to claim 19, wherein the administration route is selected from oral administration, buccal administration, mucosal administration, nasal administration, intra peritoneal administration, subcutaneous injection, intra muscular injection, transdermal administration and intravenous injection.

37 A powder formulation for enhanced bioavailability of bioactive compounds manufactured by lyophilization of the dispersion of the composition according to claim 19 by adding 0~30% (w/v) of a cryoprotectant.

38 The powder formulation for enhanced bioavailability of bioactive compounds according to claim 37, wherein said cryoprotectant is selected from the group consisting of a sugar, an amino acid and a protein.

39 The powder formulation for enhanced bioavailability of bioactive compounds according to claim 38, wherein said sugar is selected from mannitol or trehalose, said amino acid is arginine, and said protein is albumin.

40 The powder formulation for enhanced bioavailability of bioactive

compounds according to claim 37, wherein the administration route is selected from oral administration, buccal administration, mucosal administration, nasal administration, intra peritoneal administration, subcutaneous injection, intra muscular injection, transdermal administration and intravenous injection.

41 The method of preparing the powder formulation for enhanced bioavailability of bioactive compounds, wherein said method comprises the steps of:

1) dispersing the liquid formulation according to claim 19 in water to prepare the dispersion; and

2) lyophilizing said dispersion in step (1) in the presence of cryoprotectant to prepare the powder formulation.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 47/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)
CA Online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 2001068139 A1 (Korea Institute of Science and Technology) 20 September 2001 (20. 09. 2001) abstract; examples; claims.	1 - 41
Y	WO 9906043 A1 (Pharmacia & Upjohn Company) 11 February 1999 (11. 02. 1999) abstract; examples; claims.	1 - 41
Y	US 5958876 A (Novatis A.G.) 28 September 1999 (28. 09. 1999) see entire document.	1 - 41

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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